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SOME POINTS OF INTEREST CONCERNING THE COCKLEBUR AND ITS SEEDS

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The cocklebur, *Xanthium,* of which there are several species, is a coarse annual plant that attains a height of about three feet when permitted to grow in a rich, moist soil. Different species of the plant are widely distributed in many parts of the temperate zone. One authority states that more than twenty different species are found in the United States. Gray's New Manual of Botany, seventh edition, illustrates five species, but one of this number having been naturalized from South America. Nelson's Encyclopedia states that *X. canadense, X. commune, X. speciosum* and *X. echinatum* are the distinctly American species, while *X. spinosum* and *X. strumarium,* though found in this country, are indigenous to the old world. Apparently *X. commune* and *X. canadense* are the species most often found in Kentucky.

The burs of these plants are a pest of considerably more consequence in this state than is generally recognized. A rather conservative estimate shows that cockleburs cause an annual loss of at least $50,000 to the wool growers in Kentucky. In addition, much annoyance is caused by the burs becoming entangled in the farmer's clothing and in the manes and tails of horses and coats of live stock other than sheep. It is known also that the seeds of the plant contain a poisonous substance which is transmitted to the young seedlings, and live stock are sometimes poisoned from eating the plants in the two-leaf stage of their growth.

HISTORICAL

Rhodes (7) has recently shown that the seeds contain an oil which he thinks can be put to the same use as olive oil. He also states that the oil possesses drying properties which make it of value as a substitute for linseed oil.

In certain parts of Kentucky the idea is common among farmers that the color of the burs of this plant in the early fall is a good indication of the approach of killing frost. It is stated by persons who claim to have made many observations that the burs are never bitten by the frost; that they always reach maturity and turn brown a few days previous to the first appearance of frost in the autumn regardless of whether it comes early or late in the season. Therefore, so long as the burs remain green, there is no immediate danger of frost. The change from green to brown is regarded as a danger signal showing that frost is near at hand, and that no time should be lost in harvesting such crops as are likely to be injured by the frost.

While the economic possibilities of the cocklebur have only recently been suggested, the plant has long afforded a puzzle to the farmer and the scientist. The opinion appears to be widespread that one of the two paired seeds will germinate the first spring after it has matured whereas the remaining seed lies dormant until the second spring or some time thereafter.

Hunt (4), in describing the cocklebur, in his textbook on the Cereals in America, states that "Each bur contains two seeds, only one of which grows the first year, the other remaining dormant until the second year, unless the plant of the first seed has been destroyed, when, as shown by McCleur, the second seed may germinate."

The apparent dormancy in the seeds of the cocklebur has been made the subject of study and investigation by scientists in this and other countries for more than thirty years.

In 1889, Arthur (1), at the Indiana Experiment Station, began a series of experiments and observations on the delayed germination of the cocklebur and other paired seeds. Six years later in the summary of his paper on this subject he states that the common saying that one seed of a pair grows one year and the other the next is found to have no foundation when tested with seeds of oats and sand-bur, but to be essentially true when applied to the cocklebur. The cause of the difference in the action of the two seeds appears to be constitutional; a hereditary character residing in the protoplasm of the embryo. He also found that at the time the large seeds were undergoing germination processes, they contained sugars, whereas the small seeds contained none, and he came to the conclusion that there was a difference in the enzyme content of the two seeds, which played a very important rôle in the difference in time at which the two seeds germinated.

In 1898, Masterman (6), during an investigation on the sprouting of the cocklebur seeds, planted 1000 burs each year for three successive years. In the first year he observed that 917 of the burs grew two plants each, and in the second and third years that 921 and 913 burs, respectively, produced two plants each. He also states that of the remaining 249 burs, some grew one plant and others grew none. Some of the burs which grew no plants contained either one or two apparently sound seeds. He further states that he searched among specimens growing for a mile along a creek for two plants growing together and not nearer than five inches to any other plant. Of the 1500 specimens examined each year for three years, two plants usually grew from one bur.

In 1906, Crocker (2) made a study of delayed germination of the seeds of the cocklebur and the seeds of other plants, and arrived at the conclusion, with respect to the seeds of the former plant, that the cause of delayed germination was due to the seed-coat and not to a difference in the enzymes or to hereditary characters contained in the embryo of the seeds as had been suggested by Arthur. In the upper or small seed of the cocklebur the delay is due to exclusion of oxygen by the seed-coat.

In 1909, Shull (8) published a paper on the oxygen pressure and the germination of Xanthium seeds, in which he pointed out that the oxygen pressure necessary for germination is quite low and is not the same for the two seeds. Again, in 1911, he published the results of a further study on the oxygen minimum and the germination of Xanthium seeds, in which he found that the two seeds of this plant have a high oxygen demand, and the difference in this demand in the two seeds acts with the coats to secure delay and a difference in delay in the two seeds. But if the coat has been removed, the demand for oxygen by the embryo is too low to be significant in securing delay in germination.

EXPERIMENTAL

In the course of some experiments on the presence of oxidases and peroxidases in different species of seeds the idea was conceived that the paired seeds of the cocklebur might show some interesting variations in the reactions for the presence of these enzymes. A considerable quantity of cockleburs were gathered about the first of March, 1920, from plants of *Xanthium commune* (according to Gray's New Manual of Botany) that had grown in the summer of 1919. The burs averaged about 20 mm. in length by 8 mm. in diameter and were thickly covered with hooked spines which were about 4 to 5 mm. long. Each bur usually contained two seeds which were unequal in dimensions and weight. The following table contains the dimensions of ten of the large seeds and ten of the small seeds as determined by means of calipers.

TABLE I. *Dimensions of seeds in mm.*

Large Seeds			Small Seeds		
Length	Width	Thickness	Length	Width	Thickness
13.00	4.00	2.00	11.00	3.00	1.75
13.00	3.50	2.00	11.50	3.00	1.50
13.50	3.50	2.00	11.00	3.00	1.75
14.00	3.50	2.00	11.00	3.00	1.75
13.50	3.50	2.00	11.50	3.25	1.50
13.50	3.50	2.00	10.50	3.00	1.50
12.50	3.50	2.00	11.50	3.00	1,50
12.00	3.50	2.00	11.75	3.25	1.75
13.50	4.00	2.00	11,00	3.25	1.50
12.00	3.50	2.00	10,25	3.00	1.50
Av. 13.05	3.60	2.00	11.10	3.07	1.60

The average weight of 100 of the large seeds was 64.75 milligrams each. The average weight of 100 of the small seeds was 44.90 milligrams each, or a difference of 19.85 milligrams.

As the seeds were dissected from the burs they were placed alongside of each other for comparison. The large seeds were then placed in one receptacle and the small seeds in another, similar receptacle. Only sound and well developed paired seeds of apparent equal perfection were selected. After closely observing a number of the paired seeds, some points of difference other than dimensions and weight were recognized. The large seeds are flat on one side and oval on the opposite side. The small seeds are concave on the under side and oval on the top. The large seeds, at the radicle end, are pointed and have some resemblance to an arrow head. The small seeds, at this end, are curved and are less pointed.

Upon testing equal amounts of the paired seeds for oxidases and peroxidases, it was found that the former were present in only small amounts in each of the two lots of seeds, whereas a strong reaction for peroxidases was obtained in both the large and small seeds. The fact that both large and small seeds gave a strong peroxidase reaction and of equal intensity was some indication that they also possessed equal viability at the same time. In order to determine this point more definitely, equal numbers of the large and small seeds, with their seed coats intact and unbroken at any point, were planted in moist sand contained in beakers and placed in an incubator regulated at 28° C. After five or six days most of the large and small seeds had come up. After ten days 100 percent of the large seeds and 80 percent of the small seeds had germinated, thus showing that a rather high percentage of the small seeds would germinate at approximately the same time as the large seeds, under the conditions described. The results obtained on the germination of the small seeds were so markedly different from those reported by the previous investigators that a duplication of the experiment under more nearly normal conditions was made. Accordingly, 50 of the large and 50 of the small seeds were planted at the same depths in pots of moist sand containing the necessary plant-food elements for growth. The pots were kept in the greenhouse. After ten days, 100 percent of the large and 86 percent of the small seeds had germinated. Plants from the large seeds were first to make their appearance through the sand. About twenty-four hours intervened before any plants from the small seeds appeared. As the plants came up it was noticeable that the large seeds produced correspondingly large seedlings. The small seeds which did not germinate soon after they were planted have not done so after having been subjected to conditions suitable for germination for ninety days, showing that about twenty percent of the small seeds were non-viable.

This experiment demonstrates that the small seeds do not exhibit any marked degree of dormancy when they are removed from the burs, with their seed-coats intact and unbroken. It also shows that the dormancy attributed to the seeds of this species is only apparent and not real and that there is no more than twenty-four hours' difference in the time at which viable seeds, both large and small, will germinate, under the conditions mentioned. Therefore there is no inherent property contained in the embryos of the small seeds of this species which is the cause of delayed germination, neither is there any evidence that the seed-coat in any way interferes with germination.

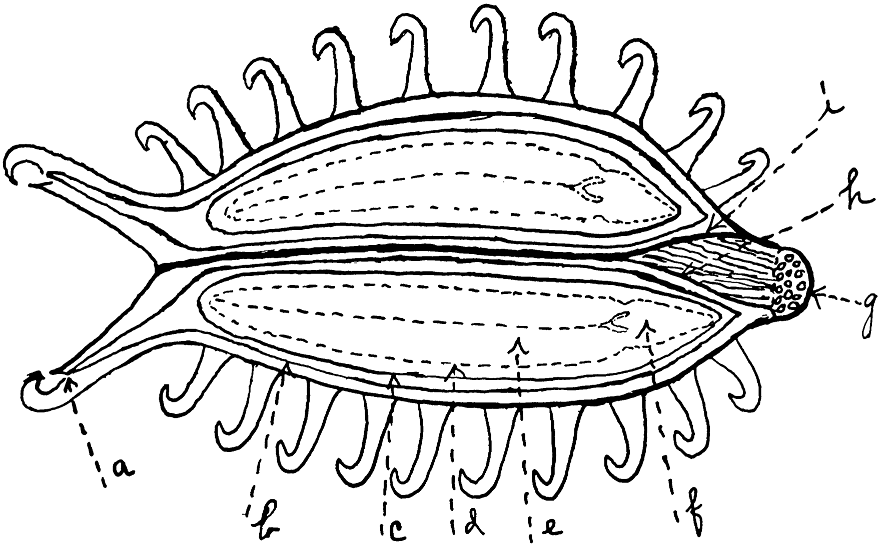

FIG. 1. Longitudinal section through a bur of *Xanthium commune*. *a,* style; *b,* embryo sac; *c,* seed coat; *d,* endosperm; *e,* cotyledons; *f,* embryo; *g,* stem; *h,* minor arm of septum; *i,* major arm of septum.

In order to determine if there was any cause contained within the bur which was responsible for the apparent dormancy, one hundred normal burs were planted in moist sand and kept in the greenhouse. After about ten days most of the burs had produced only one plant each, and after more than ninety days only five percent had produced two plants. A number of the burs in which only one seed had germinated were removed and examined to determine if a second seed remained in the bur and if it apparently was in a sound condition. It was found that in such burs as had contained two well-matured seeds, only one of which had germinated, it was always the larger or lower seed which was first to produce a plant. Ten of the small seeds, after having lain in the bur for sixty days under conditions favorable for the germination of the large seeds, were removed from the bur and planted in moist sand. Nine plants grew from these seeds within ten days, thus showing that the apparent cause of dormancy was due to conditions within the bur and not in the seed-coat or embryo.

Longitudinal sections were made at a point that divide the bur and seeds into equal parts, and the mechanism of the different parts examined by means of a hand lens. The accompanying drawing, figure 1, which was made on a scale about five times the size of normal burs, shows the various parts of the bur and seeds and the relations between those parts. It will be seen that the radicle end (right in figure) of the lower or large seed lies much nearer the base of the bur than the radicle end of the small seed. Apparently, then, the large seed is more readily accessible to the water and oxygen that is necessary for germination than the small seed. It will also be noted that the septum which divides the bur into an upper and a lower compartment separates into a Y-form near the radicle end of the two seeds, as shown by the letters "*h*" and "*i*" in figure 1. After examining the "*h*" and "*i*" parts of the septum in a number of burs, it was found that in most cases the "*i*" arm of the Y was shorter and much stiffer than the "*h*" arm. The end of the "*i*" arm fits very closely against the inner wall of the bur and therefore undoubtedly prevents both water and oxygen from reaching the base of the small seed to a much greater degree than is the case with the large seed. These differences can be readily seen in figure 1. The more protected condition of the small seed as compared with the large seed is the cause of the apparent dormancy in the seeds of this particular species. The large seed, being more readily accessible to available oxygen and moisture, receives these necessary elements when the conditions for germination maintain, whereas, it is necessary for the "*i*" arm of the Y septum to disintegrate, in part or whole, before the moisture and oxygen required for germination can reach the small seed. Therefore the large seeds germinate first together with a few of the small ones which, in spite of their protection, receive enough moisture and oxygen for germination. Hence we have all of the viable large seeds, and only a few of the corresponding paired small seeds, producing plants the first season after maturity. By the second time natural conditions have become favorable for germination the "*i*" part of the Y septum will have disintegrated to such an extent that the small seeds receive all of the elements necessary for germination. Therefore a crop of plants from the small seeds appears the second spring after the seeds mature.

Masterman's observations of cockleburs in which most of the burs produced two plants the first spring after maturity, suggests that in some species of *Xanthium* the mechanism of the bur may be different from that which exists in the burs of *Xanthium commune*.

Observations were made in the early spring of 1920 on burs that lay on the ground, where they had fallen from plants which grew in the previous year. About the first of May burs lying uncovered were putting forth radicles which penetrated the ground at right angles to the bur, and, as the plant developed, the cotyledons were withdrawn from the cavity which they had occupied. But few of the burs produced two plants at the same time, under natural conditions, and one of the two plants which had grown from the same bur was larger than its mate. Apparently, then, where a large number of the plants grow close together the small twin plants would be soon outgrown and shaded to such an extent that but few would produce seeds. Thus, under crowded conditions, the plants from the large seeds are the ones that propagate the species.

The difference in the size and vigor of the plants from the large and small seeds naturally called for some explanation as to the cause. Under normal conditions of plant growth, the principal limiting factors are moisture, temperature, available plant food and physiological well being. Since the factors of moisture and temperature were controlled, it therefore appears that the difference in the size and vigor of the two different plants is either a question of available plant food during the germination and early stages of the growth of the seedling or of inherent factors of heredity.

In order to determine more definitely what differences exist in the amounts of available plant food in the paired seeds, an analysis of the ash and some of the organic constituents of the seeds was necessary. Accordingly, 25 grams each of the large and small seeds of the species previously mentioned were ashed and the mineral constituents determined. The sulfur, nitrogen and oil were determined in other portions of material.

TABLE II. *Mineral and some of the organic constituents of the large and small seeds of the cocklebur (Xanthium commune), calculated on the water-free material*

	Large seeds—percent	Small seeds—percent
Ash	6.5629	6.3453
Silica	.0145	.0139
Iron oxid	.0075	.0070
Manganese sesquioxid	.0081	.0094
Calcium oxid	.1186	.1108
Magnesium oxid	1.2468	1.2366
Potassium oxid	1.3495	1.2949
Sodium oxid	.2028	.2397
Phosphorus pentoxid	3.3781	3.3241
Sulfur (total)	.5891	.5250
Nitrogen	8.5736	8.2545
Protein	52.8850	51.5906
Oil	28.1621	29.6118

From the results shown in Table II it appears that the large seeds contain slightly more mineral matter in every case except manganese and sodium. In regard to manganese, this can readily be accounted for by the fact that the writer (5) has previously shown that the seed coat contains more manganese than an equal amount of the cotyledons. On the average it requires 1.443 small seeds to equal the weight of one large seed; it is readily apparent then that more seed-coat material will be contained in the small seeds when equal weights of the two seeds are taken. No significance is attached to the slightly larger amount of sodium contained in the analyses of the small seeds.

Since the two paired seeds are unequal in size, a comparison of the amount of each essential plant food element contained in an equal number of the two kinds of seeds is of some interest in connection with the fact that we have obtained different sized seedlings from the paired seeds.

From the average weight of the large and small seeds the amount of oil, ash and each important plant food element contained in an equal number of the large and small seeds are given in Table III.

TABLE III. *Ash, oil and mineral matter contained in equal numbers (100) of the large and small seeds*

	Large seeds—grams	Small seeds—grams
Ash	.3868	.2569
Silica	.0009	.0006
Iron oxid	.0004	.0003
Manganese sesquioxid	.0005	.0004
Calcium oxid	.0070	.0046
Magnesium oxid	.0711	.0505
Potassium oxid	.0792	.0530
Sodium oxid	.0120	.0099
Phosphorus pentoxid	1.9781	1.3588
Sulfur (total)	.0898	.0550
Nitrogen	.5261	.3476
Oil	1.6590	1.2178

From the results contained in Table III it is evident that there is a considerable difference in the amount of plant food available for seedlings produced from the large and small seeds. Apparently, then, the amount of plant food contained in the paired seeds of this species of plant is a factor which determines the size and vigor of the seedlings produced from each of the paired seeds. In every respect the plants from the large seeds are larger and more vigorous than those from the small seeds. This difference still maintains in plants from the two different seeds after more than sixty days of growth under the same conditions. This fact further emphasizes the importance of the selection of the largest and heaviest seeds intended for the propagation of the more useful species (3), especially when comparative experimental data are to be obtained by plant growth with fertilizers. It will be observed that the small or upper seeds have shown a weaker viability than the large or lower seeds. In well-selected large and small seeds the former will have a viability of from ninety to one hundred percent and the latter from eighty to ninety percent, thus indicating a relationship existing between viability and the size of the seeds.

Arthur states that he found no sugars in the small seeds but a considerable amount in the large seeds. To determine if this condition maintained in the seeds of the species under examination, five grams each of the large and small seeds were ground fine separately and transferred to two dry, 100 c.c. volumetric flasks. Each of the flasks was filled two thirds full of distilled water and shaken vigorously at intervals during two hours, after which the volumes were made to the mark and their contents poured into dry folded filters. An aliquot of each of the filtrates was taken, tested directly for reducing sugars and none were found in either the large or small seeds. Other aliquots were transferred to flasks and hydrolyzed with dilute sulfuric acid, neutralized and transferred to a boiling Fehling's solution. The filtrates from each of the two portions of seeds when treated as described reduced Fehling's solutions, thus showing that each of the seeds contained a glucoside. The percentages of reducing sugar produced, calculated as glucose, were 5.32 for the larger and 5.11 for the smaller seeds, on the water-free basis. Other large and small seeds were allowed to germinate until the radicles were about two inches long and the cotyledons had opened out in a horizontal position. Definite weights of the seedlings at this stage of their growth were then treated for sugar in the same way as the dormant seeds described above. It was found that the total reducing sugar after hydrolysis had increased nearly one hundred percent in each of the seeds during the time germination had progressed to the point indicated. This point is of interest, because of the rate at which the sugar-forming substance was produced and also because the seeds of this plant contain no starch; therefore the sugars formed must have been derived from non-starchy carbohydrates, *i.e.,* fat or cellulose. This increase in the sugar-forming substance during germination may also indicate an increase in the glucoside content and if so this may account for the poisonous effect of the young seedlings when live stock eat them. Qualitative tests carried out for the presence of an alkaloid gave negative results.

The presence of oxidase, peroxidase, catalase, proteolytic and lipoclastic enzymes was demonstrated by appropriate tests on both large and small seeds but no very positive reactions were obtained for the presence of amylase. The catalase activity was not very marked, one gram of the powdered seeds producing no more than 20 c.c. of gas in 10 minutes, from either large or small seeds, whereas an equal amount of other seeds having a strong catalase reaction will liberate as much as fifty times this volume of gas under the same conditions.

Microscopical examinations of mounted sections showed that there is no material difference in the cell structure of the large and small seeds. The seeds are made up of four different types of cells. The cell structure of the seed coats from the large and small seeds was practically identical—hence there can be no good reason for believing, from the standpoint of structure, that the coat on the small seed would in any way prevent germination to a greater degree than the coat on the large seed.

The cocklebur is the source of considerable economic loss to the farmers of Kentucky and it appears that the possibility of obtaining a commercial product from the plant that would compensate for the damage done to the wool crop is remote. There are no good reasons for allowing the plant to propagate its kind, and effective measures should be taken to bring about its extermination.

CONCLUSIONS

1. The dormancy which has hitherto been attributed to one of the seeds of this species of plant is only apparent and not real.
2. When well developed, paired seeds are removed from the burs and subjected to conditions suitable for germination they will germinate at approximately the same time. The mechanical conditions within the bur are the cause of delayed germination in the smaller cocklebur seeds.
3. The large seeds produce larger and more vigorous plants than the small seeds.
4. The chemical composition and the cell structure of the two seeds are similar.

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